Soil Fungi Respond More Strongly than Fine Roots to Elevated CO$_2$ in a Model Regenerating Longleaf Pine-Wiregrass Ecosystem

M. Luke McCormack,[1] Seth G. Pritchard,[2]* Sabrie Breland,[2]
Michael A. Davis,[3] Stephen A. Prior,[4] G. Brett Runion,[4]
Robert J. Mitchell,[5] and Hugo H. Rogers[4]

[1]Department of Horticulture, Pennsylvania State University, University Park, Pennsylvania 68022, USA; [2]Department of Biology, College of Charleston, Charleston, South Carolina 29401, USA; [3]Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, Mississippi 39406, USA; [4]USDA-ARS National Soil Dynamics Laboratory, 411 South Donahue Drive, Auburn, Alabama 36832, USA; [5]Joseph W. Jones Ecological Research Center, Newton, Georgia 31770, USA

ABSTRACT

Rising atmospheric CO$_2$ concentration will affect belowground processes and forest function. However, the direction and magnitude of change for many soil processes are unknown. We used minirhizotrons to observe fine root and fungal dynamics in response to elevated CO$_2$ in a model regenerating longleaf pine community in open-top chambers. The model community consisted of five plant species common to xeric sandhills longleaf pine stands: *Pinus palustris*, *Quercus margaretta*, *Aristida stricta*, *Crotalaria rotundifolia*, and *Asclepias tuberosa*. Elevated CO$_2$ significantly increased both fine root and mycorrhizal tip standing crop by more than 50% in the deeper soil horizon (17–34 cm). Rhizomorph standing crop was nearly doubled in both deep and shallow soil ($P = 0.04$). Survivorship results for fine roots and rhizomorphs varied between soil depths. Fine root survivorship was likely influenced more by changes in community composition and species interactions driven by elevated CO$_2$ rather than by direct effects of elevated CO$_2$ on the fine roots of individual species. In this system, it appears that elevated CO$_2$ led to a greater reliance on fungal symbionts to meet additional nutrient requirements rather than substantially increased root growth.

Key words: elevated CO$_2$; fine roots; mycorrhizal fungi; longleaf pine; wiregrass; minirhizotron.

INTRODUCTION

Fossil fuel emissions and land-use change have driven significant increases in atmospheric CO$_2$ over the past century (Meehl and others 2007). During this time, temperate trees and forest soils have played a key role in the global C cycle acting as a large C sink (Dixon and others 1994; Goodale and others 2002; Lal 2005). However, the potential

for temperate forests and forest soils to sequester C in the coming decades is a major source of uncertainty in most models addressing future climate scenarios (Friedlingstein and others 2006; Schimel and others 2001).

Globally, soil organic C pools hold approximately three times more C than the atmospheric pool and four times more C than is contained in biota (Lal 2004). Acting as a dynamic biogeochemical coupler, fine roots and mycorrhizal fungi are the major conduit by which water and nutrients are taken in by plants and represent a major pathway by which C moves into the soil (Vogt and others 1990; Richter and others 1999). Changes in the standing crop, production, mortality, and survivorship of both fine roots and mycorrhizal fungi can, therefore, directly affect the function and net primary productivity (NPP) of an ecosystem as well as moderate its potential for long-term C storage (Norby and Jackson 2000). Understanding the belowground response to global change factors, particularly properties such as fine root and soil fungal turnover rates, which are notoriously difficult to measure, will be critical if we hope to predict responses of ecosystems to continued atmospheric CO_2-enrichment.

Using a cost:benefit approach, Eissenstat and others (2000) hypothesized that elevated CO_2 would result in an overall increase in fine root lifespan for a single species. Increased longevity might be expected to follow from the increase in carbohydrates available to support root maintenance and protection, for instance, or from the reduction in tissue N concentration in fine roots grown under CO_2-enriched conditions. Furthermore, exposure to atmospheric CO_2-enrichment has also been shown to increase fine root diameters (Milchunas and others 2005) and to preferentially stimulate fine root proliferation in deeper soil (Norby and others 2004). Fine root diameters, depth of roots in soil, and carbohydrate content are all predicted to correlate positively with fine root longevity (for example, Guo and others 2008). In spite of frequent conjecture about the likely effects of CO_2-enrichment on fine root longevity, few data on this aspect of plant function have been reported. And within intact forests or model ecosystems where plants must compete with one another for a common pool of soil resources, predictions become more tenuous and acquiring relevant empirical data even more difficult. In one of the few studies to quantify fine root dynamics in an intact forest Norby and others (2004) found little evidence of a CO_2 effect on root lifespan in a sweetgum forest. Pritchard and others (2008a) observed a moderate

decrease of median lifespan from 574 days in ambient plots to 500 days in CO_2-enriched plots within an intact loblolly pine forest. Although both sites are well characterized, it would be difficult to attribute changes in root dynamics to a single species, or to a shift in species composition, due to the scale of the design and species diversity within plots.

To our knowledge, no experiments have characterized both fine roots and associated fungi in a forest community in a single effort. However, evaluating each group separately can lead to limited or incorrect interpretations regarding forest potential for nutrient and water uptake. For example, Rillig and others (1999) found differing patterns of allocation to fine roots and arbuscular mycorrhizal fungi in two separate grasslands exposed to elevated CO_2. One grassland responded by increasing fine root production, while the production of mycorrhizal fungi remained static. In the second grassland, fine roots showed little response to elevated CO_2, while production of mycorrhizal fungi increased significantly. As fine roots and mycorrhizal fungi may respond differently depending on several factors, including species composition and soil fertility, it is important to assess both of their responses in tandem in an effort to better gauge whole-system response to elevated CO_2.

Our goal in this study was to quantify CO_2 induced changes in production and longevity of fine roots and soil fungi including mycorrhizae and extraradical rhizomorphs. This was done in a system where competitive interactions were allowed to take place and the competitive success of individual species could be inferred based on whole plant growth and individual mortality reported previously from this study site (see Davis and others 2002; Runion and others 2006). In the end, changes in species assemblages that follow from exposure to CO_2-enrichment may have a greater effect on fine root and soil fungal dynamics than might be predicted based on effects of CO_2-enrichment on fine roots of individual species and their associated soil fungi grown in isolation.

We conducted our study using a model regenerating longleaf pine-wiregrass community grown under ambient (365 µl l^{-1}) and elevated (720 µl l^{-1}) CO_2 in open-top chambers (OTC) for 3 years. Our community consisted of longleaf pine (*Pinus palustris* Mill., evergreen), wiregrass (*Aristida strictaI* Michx., C4 grass), sand post oak (*Quescus margaretta* Ashe, deciduous tree), rattlebox (*Crotalaria rotundifolia* Walt. ex Gemel, C3 perennial herbaceous legume), and butterfly weed (*Asclipias tuberosa* L., herbaceous

C3 dicotyledonous perennial). Fine root (≤ 2 mm diameter), rhizomorph, and mycorrhizal tip dynamics were recorded for 2.5 years using minirhizotrons.

MATERIALS AND METHODS

Model Longleaf-Wiregrass Community

Community construction has been previously described (see Davis and others 2002; Pritchard and others 2001). Briefly, a model regenerating longleaf pine-wiregrass community was constructed at the National Soil Dynamics Laboratory in Auburn, Alabama, United States. An outdoor soil bin (2 m deep $\times$ 6 m wide $\times$ 76 m long) was filled in 1970 with a Blanton loamy sand (loamy, siliceous, thermic Grossarenis Paleudults) taken from a typical Southeastern Coastal Plains longleaf pine area (Tuskegee National Forest in Macon County, Alabama). During early spring, 1998, the soil bin was divided into 0.75 m^2 quadrats containing 16 equally spaced planting positions. Individuals of five plant species common to a xeric sandhills longleaf pine ecosystem were randomly located in 10 of the 16 planting positions reflecting natural densities of each species within the community: three longleaf pine (*Pinus palustris* Mill.), an evergreen; three wiregrass (*Aristida stricta* Michx.), a C4 grass; two sand post oak (*Quercus margaretta* Ashe), a deciduous tree; one rattlebox (*Crotalaria rotundifolia* Walt. ex Gmel.), a C3 perennial herbaceous legume; and one butterfly weed (*Asclepias tuberose* L.) an herbaceous C3 dicotyledonous perennial.

Beginning in June 1998, 12 open-top chambers 3 m in diameter were used to deliver ambient (365 µl l^{-1}) and elevated (720 µl l^{-1}) levels of CO_2 using a delivery system described by Mitchell and others (1995). The soil bin was divided into six blocks and CO_2 treatments were randomly assigned within each block. During the summer of 1998 plants were regularly irrigated using a drip irrigation system to encourage plant establishment. Any plants that died during the first 2 months were replaced to compensate for losses due to transplanting. Afterwards, plants that died were not replaced and it was assumed death was not due to transplanting. Beginning in August 1998, irrigation ceased and plots received only ambient rainfall to more accurately mimic conditions of a xeric sandhills longleaf ecosystem.

Minirhizotron Images

Minirhizotron tubes were installed in May 1998. Two tubes were oriented with the tube opening facing the chamber door (24 tubes total). Each tube was installed at a 45° angle to a vertical depth of approximately 34 cm. The tubes were placed so that the point where they entered the ground was 1.2 m away from the sides of the chamber ring and the maximum distance of the tubes away from the chamber edge was approximately 1.35 m. At the termination of the experiment, some of the longleaf pine seedlings were near the top of the chambers (that is, a maximum height of < 2.4 m). It is unlikely that seedlings from outside the chambers contributed significantly to root colonization along the minirhizotron tube/soil interface. The aboveground portion was covered with a closed-cell polyethylene sleeve and sealed with a rubber cap to prevent light intrusion and minimize heat exchange between aboveground air and belowground portions of the tube. A PVC cap was then installed over the rubber cap and sleeve to protect the tube and act as further insulation. To prevent shifting of minirizotron tubes they were attached to aluminum clamps and anchored in the ground using 40 cm steel rods. Tubes were installed at the same time plants were transplanted into the plots. Images were not collected for 4 months after installation to allow sufficient time for roots of recently transplanted species to contact the tube surfaces.

Image collection began in September 1998, using a BTC-100$\times$ microvideo camera (Bartz Technology Corp., Carpinteria, CA). The camera was equipped with an indexing handle and allowed for repeated imaging of precise locations within the tubes (Johnson and Meyer 1998). Video frame images were taken at approximately 4-week intervals a total of 33 times between September 1998 and March 2001. Video frames were later replayed and every other frame was digitized in the laboratory. Data were recorded (number of roots/rhizomorphs, length, diameter, and color) for all live and dead roots and rhizomorphs in each frame (18 frames per tube) within each tube and for all sessions (> 12,000 images total) using RooTracker software (Dave Tremmel, Duke University Phytotron). Standing crop was quantified as the total number/ length of a given structure that was considered live at a given sampling point. Production was quantified as the length/number of structures that developed since the preceding sampling date and mortality was the length/number of structures that disappeared or appeared to become nonfunctional during a given sampling interval. These same criteria were applied to mycorrhizal root tips and rhizomorph structures. We assumed that all root and fungal structures we observed originated from within each treatment plot. However, because

there were no physical partitions in the soil bin separating plots it is possible that there was some intrusion of roots and soil fungi that originated from areas outside the OTCs.

Roots were classified as dead when they became structurally fragmented, shriveled to approximately half original diameter or disappeared. Rhizomorphs were classified as dead when they shrank to less than half the original diameter or disappeared, but were left classified as alive when minor fragmenting occurred. Due to difficulties inherent in classifying roots and rhizomorphs as alive or dead using visual cues alone it is possible that some roots and rhizomorphs may have been classified as alive when they were functionally dead and vice versa. The overall error is likely to be small but should not impact differences observed between treatments.

To investigate potential differences associated with root and rhizomorph diameter, we divided the fine root pool into two diameter classes (<0.5 mm and > 0.5 mm) and rhizomorphs into two diameter classes (<0.2 mm and >0.2 mm). Maximum diameter of a given structure during its lifespan was used in this analysis. We then analyzed the effects of elevated CO_2 and different soil horizons on the different diameter classes.

Ectomycorrhizal tips, which are visually distinct from non-mycorrhizal root tips, were also counted in each image and followed through time from which standing crop, production, and survivorship data were calculated. Tips were classified as alive at first appearance and as dead once they shriveled to approximately half of their original size, turned black and faded until they were no longer recognizable, or disappeared. Production and standing crop of ectomycorrhizal root tips were quantified as described above for fine roots and rhizomorphs.

Statistics

The experimental design was a split block with two CO_2 treatments (elevated and ambient) randomized with six replications. Each of the 12 chambers contained two subsample minirhizotron tubes. In all cases, plot means were used for statistical analyses. During the analysis the soil profile was broken into two classes. The "shallow" horizon represented images from an approximate depth of 0–17 cm, or the upper half of the total depth of our minirhizotron tubes. The "deep" horizon represented images covering the lower half of the minirhizotron tubes (that is, a depth of 17–34 cm). Data were analyzed using a linear mixed model repeated measures approach with time as the repeated variable (proc mixed, SAS Corporation, Inc., Cary, NC, USA); CO_2

treatment and depth were treated as fixed effects and block was the random effect. We used the compound symmetry covariance structure because it yielded the best fit with respect to Akaike's Information Criterion (AIC). In addition to this time series approach, we also summed production and mortality for the entire experiment duration to determine treatment effects on cumulative production/mortality.

To characterize and compare root and fungal (rhizomorph and mycorrhizal tip) longevity, we used the Kaplan–Meier method to estimate survivorship for each treatment combination and Cox's proportional hazards model to test for main effects of treatment, depth, and diameters as well as their interactions (Kaplan and Meier 1958). Log-rank and Wilcoxon's tests were further used to compare homogeneity of survivorship curves between groups of interest identified from Cox's test. All root and fungal structures that were present at the beginning of the study and those that remained at the end were considered censored. All statistical analyses of survivorship were conducted using JMP version 7.0 (SAS Institute, Cary, NC, USA). Due to variability inherent to minirhizotron studies, significant differences were designated when $P < 0.05$ and statistical trends when $0.05 < P < 0.15$ (Steel and others 1997; Pritchard and others 2008a, b).

RESULTS

Fine Root Dynamics

Fine root production and mortality in the shallow soil horizon (0–17 cm) exceeded that in deeper soil (17–34 cm) (Tables 1 and 2). Although we observed no main effect of CO_2-enrichment, there was a significant $CO_2 \times$ depth interaction with elevated CO_2 increasing standing crop, production, and mortality by 59, 77, 81% in the deep horizon and decreasing them by 32, 18 and 16% in the shallow horizon (Table 2; Figures 1 and 2). The increased fine root standing crop in deeper soil tapered off during the last 6 months of the experiment due primarily to higher mortality during the final winter and spring of the study in CO_2-enriched plots (Figure 2).

There was a trend indicating that fine roots in ambient plots lived longer (median longevity = 562 days) compared to those in elevated plots (median longevity = 477 days) ($P = 0.13$; Table 3, Figure 3). Median longevity was 29 and 6% lower in CO_2-enriched compared to ambient plots in shallow and deeper soil horizons, respectively (data not shown). However, there was not a significant

Table 1. Summary of Repeated Measures Analyses (*P* Values Shown) for Effects of Session, CO_2 (Elevated or Ambient), and Soil Depth (0–17 vs. 17–34 cm) on Fine Root Length, Production, Length Mortality, and Length Standing Crop; Rhizomorph Length Production, Length Mortality, and Standing Crop Length; and Mycorrhizal Root Tip Production and Standing Crop over a 2.5 Year Period

Source	Production	Mortality	Standing crop
Fine root length (m m^{-2})			
CO_2	NS	NS	NS
Depth	0.02	0.07	NS
$CO_2 \times$ depth	0.04	0.05	0.0001
Session	0.0001	0.0001	0.0001
$CO_2 \times$ session	NS	NS	NS
Depth $\times$ session	0.0001	NS	NS
$CO_2 \times$ depth $\times$ session	NS	NS	NS
Rhizomorph length (m m^{-2})			
CO_2	NS	NS	0.04
Depth	NS	NS	0.003
$CO_2 \times$ depth	NS	0.09	NS
Session	0.0001	0.0001	0.0001
$CO_2 \times$ session	NS	0.09	NS
Depth $\times$ session	NS	0.07	NS
$CO_2 \times$ depth $\times$ session	NS	NS	NS
Mycorrhizal root tips (# per frame)			
CO_2	0.056	–	0.131
Depth	NS	–	NS
$CO_2 \times$ depth	0.010	–	0.005
Session	0.0001	–	0.0001
$CO_2 \times$ session	NS	–	NS
Depth $\times$ session	0.116	–	0.067
$CO_2 \times$ depth $\times$ session	NS	–	NS

Table 2. Mean ($\pm$SE) Standing Crop, Production, and Mortality for Fine Roots, Rhizomorphs, and Mycorrhizal Root Tips Observed in Control and CO_2-enriched Plots at Two Soil Depths

	Ambient [CO_2]		Elevated [CO_2]	
	0–17 cm	17–34 cm	0–17 cm	17–34 cm
Fine root length[1]				
Production	1.90 (0.31)	0.85 (0.31)	1.61 (0.31)	1.50 (0.31)
Mortality	1.52 (0.29)	0.75 (0.29)	1.32 (0.29)	1.36 (0.29)
Standing crop	731 (179)	510 (179)	553 (179)	813 (179)
Rhizomorphs				
Production	1.10 (0.33)	1.78 (0.29)	1.65 (0.29)	1.61 (0.29)
Mortality	0.66 (0.22)	1.21 (0.22)	1.15 (0.22)	0.93 (0.22)
Standing crop	251 (79)	362 (79)	538 (79)	600 (79)
Mycorrhizal root tips[2]				
Production	0.71 (0.13)	0.54 (0.13)	0.76 (0.13)	1.18 (0.13)
Standing crop	0.41 (0.07)	0.42 (0.07)	0.43 (0.07)	0.69 (0.07)

[1]*Production values for fine roots and rhizomorphs are in units of length (m m^{-2} day^{-1}).*
[2]*Units for root tips are numbers of new tips per frame per sampling interval (production) and average number of mycorrhizal root tips per frame over the course of the experiment (standing crop).*

$CO_2 \times$ horizon interaction. As expected, there was a strong effect of root diameter and soil depth on survivorship (Table 3; Figure 3). Larger diameter roots (>0.5 mm) had a 36% longer median lifespan than smaller diameter roots. The median lifespan of fine roots in the deep horizon was 32% greater than
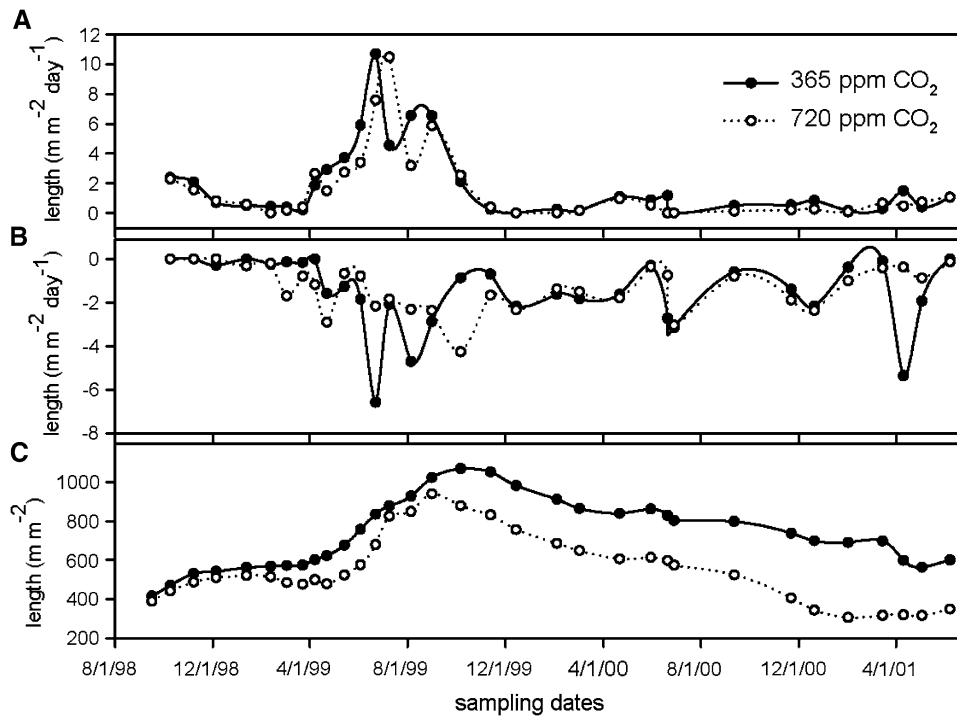
Figure 1. Fine root production (**A**), mortality (**B**), and standing crop (**C**) in the upper soil horizon (0–17 cm) across 33 image collection dates between September 1998 and March 2001.
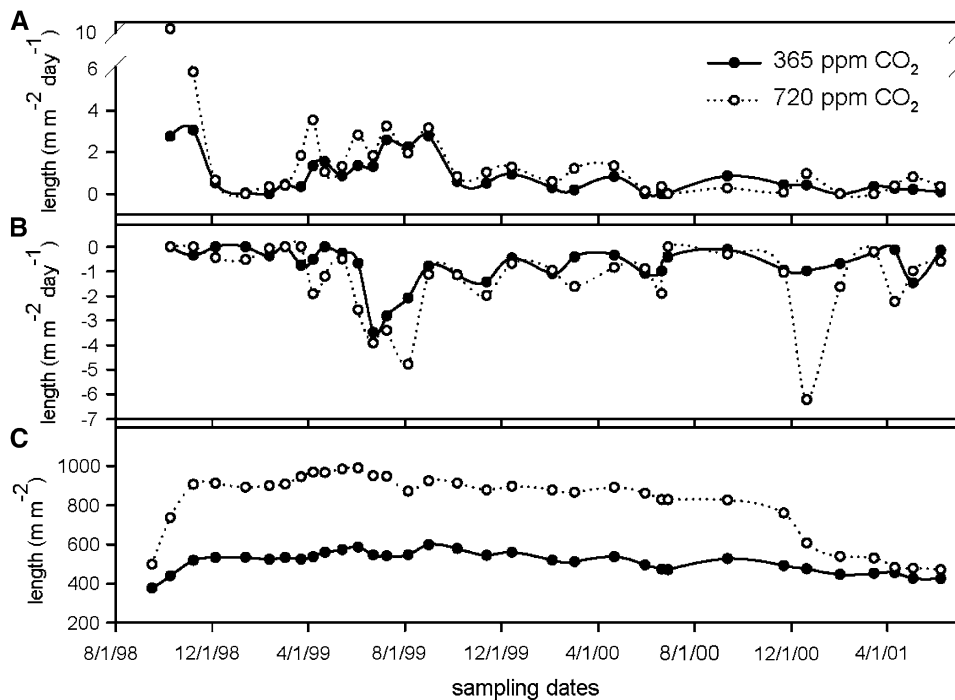

Figure 2. Fine root production (**A**), mortality (**B**), and standing crop (**C**) in the lower soil horizon (17–34 cm) across 33 image collection dates between September 1998 and March 2001.

those produced in the shallow horizon. There was also a significant effect of depth ($P = 0.003$) and a $CO_2 \times$ depth interaction for median fine root diameter ($P = 0.09$; Figure 4). Roots tended to be coarser in shallow compared to deep soil but this effect was only significant in control plots because CO_2-enrichment resulted in finer roots in shallow soil (Figure 4, *inset*). In general, variance of fine root diameters was greater in shallow than deep soil.

Table 3. Summary of Proportional Hazards Fit for Effects of CO_2 (Elevated vs. Ambient), Soil Horizon (0–17 vs. 17–34 cm), and Diameter on Rhizomorph, Fine Root, and Mycorrhizal Root Tip Survivorship

Source	Rhizomorphs			Fine roots			Mycorrhizal tips		
	df	χ^2	$P > \chi^2$	df	χ^2	$P > \chi^2$	df	χ^2	$P > \chi^2$
CO_2	1	0.01	NS	1	2.34	0.13	1	7.12	0.008
Horizon	1	3.24	0.07	1	12.7	0.0004	1	10.7	0.001
Diameter	1	4.92	0.03	1	11.3	0.0008	–	–	–
$CO_2 \times$ horizon	1	0.07	NS	1	0.21	NS	1	1.11	NS
$CO_2 \times$ diameter	1	8.06	0.005	1	0.11	NS	–	–	–
Horizon $\times$ diameter	1	4.24	0.04	1	1.93	NS	–	–	–
$CO_2 \times$ horizon $\times$ diam	1	0.89	NS	1	0.52	NS	–	–	–

Significant interactive effects were further explored with Log-Rank and Wilcoxon's tests derived from Kaplan–Meier survivorship estimates. These results are presented in Figures 3, 7, and 10.

Rhizomorph and Mycorrhizal Tip Dynamics

Overall there was significantly greater rhizomorph standing crop in deeper soil than in shallow soil ($P = 0.003$, 21% greater) along with non-significant 24 and 18% increases in production and mortality, respectively (Tables 1, 2). CO_2-enrichment increased rhizomorph standing crop by 90% ($P = 0.04$) compared to ambient plots. This result was generally conserved throughout the experiment and at both soil depths (Figures 5C and 6C). There was an interesting difference in how elevated CO_2 impacted rhizomorph dynamics in the two soil horizons. A trend was detected indicating a $CO_2 \times$ depth interaction for rhizomorph mortality with an overall decrease of 29% in the deep horizon and increase of 73% in the shallow horizon with elevated CO_2 ($P = 0.09$; Figures 5, 6). Although not significant, rhizomorph production in the deep soil horizon was also generally lower with elevated CO_2 (-10%, NS) and greater ($+51\%$, NS) in the shallow horizon. Restated, there was decreased mortality and little change in production in deeper soil, whereas there was increased mortality and increased production in shallow soils. Both strategies resulted in higher standing crop with elevated CO_2 in the shallow and deep soil horizons. However, the differences in mortality and production in shallow and deep soils suggest functional differences in the rhizomorphs at each depth. Due to the contrasting responses of each soil horizon the end result was only moderately increased production and mortality overall, 13 and 11%, respectively (NS, Table 2).

We observed a significant $CO_2 \times$ diameter effect on rhizomorph survivorship ($P = 0.005$; Table 3).

Smaller diameter rhizomorphs had significantly lower survivorship under elevated CO_2 than under ambient conditions (Figure 7A). Conversely, larger diameter rhizomorphs generally lived longer under elevated CO_2 relative to ambient conditions (Figure 7B). Longevity was greater in shallow soil for larger diameter rhizomorphs (horizon $\times$ diameter, $P = 0.04$), whereas survivorship for the smallest diameter rhizomorphs (that is, <0.2 mm) did not vary with soil depth. Median longevity of rhizomorphs was negatively related to diameter (Figure 8, $R^2 = 0.65$). We observed no effect of CO_2 or horizon on mean and median rhizomorph diameter (data not shown).

Elevated CO_2 increased mycorrhizal tip standing crop and production by 47 and 63%, respectively ($P = 0.06$ and $P = 0.13$; Table 1). Mycorrhizal tip production in elevated CO_2 plots was characterized by more frequent production events that were typically greater in magnitude with more tips produced at a given time and location than those in ambient plots. This was particularly true in deeper soil (Figure 9). The overall increases in mycorrhizal tip standing crop and production with elevated CO_2 was the result of a larger increase in mycorrhizal tip production in deeper soil. Here, the standing crop and production increased by 101 and 159%, respectively (Table 1 and 2). Finally, survivorship of mycorrhizal tips was significantly lower in shallow soil compared to deep soil. There was also a significant effect of elevated CO_2 in deeper soil which decreased survivorship of mycorrhizal tips by 25% (Figure 10). Overall, tips in deep soil turned over more slowly than those in shallow soil (172 vs. 137 mean days) and tips in ambient plots turned over more slowly than those in elevated plots (185 vs. 139 mean days).
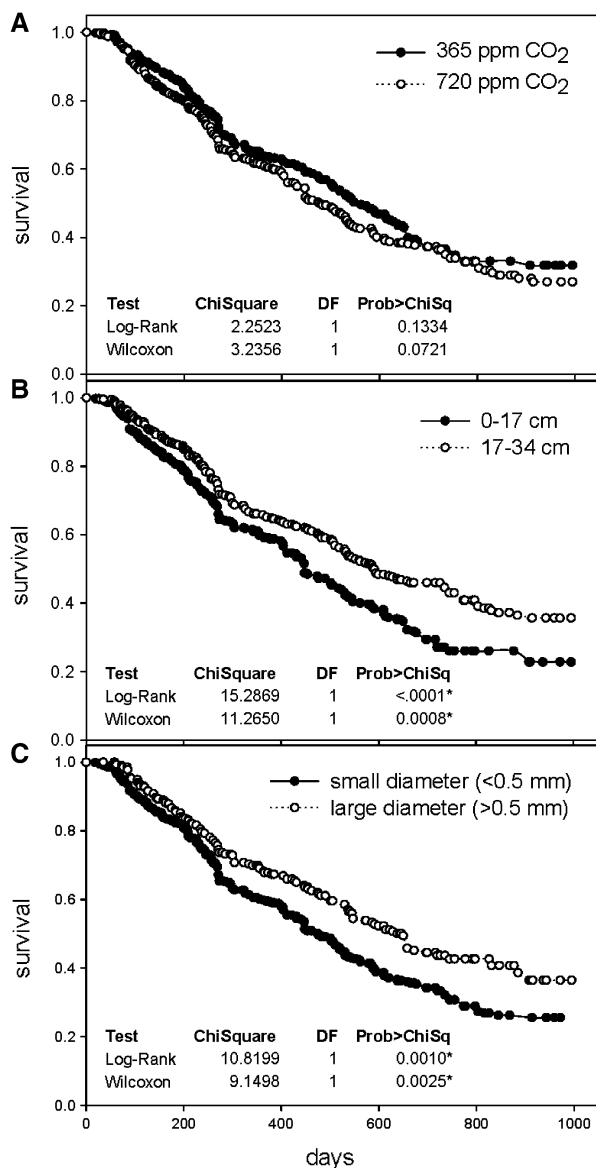
Figure 3. Kaplan–Meier survivorship curves for fine roots displaying main effects of elevated CO_2 (**A**), soil depth (**B**), and root diameter (**C**) on fine root longevity. Results of Log-Rank and Wilcoxon's tests are shown.

Discussion

Survivorship and Community Interactions

In this study CO_2-enrichment resulted in an 18% decrease in fine root survivorship. However, this was likely not a direct result of CO_2-enrichment on specific metabolic processes controlling fine root longevity. Rather, this observation is more likely attributable to increased mortality and decreased dominance of wiregrass in CO_2-enriched plots
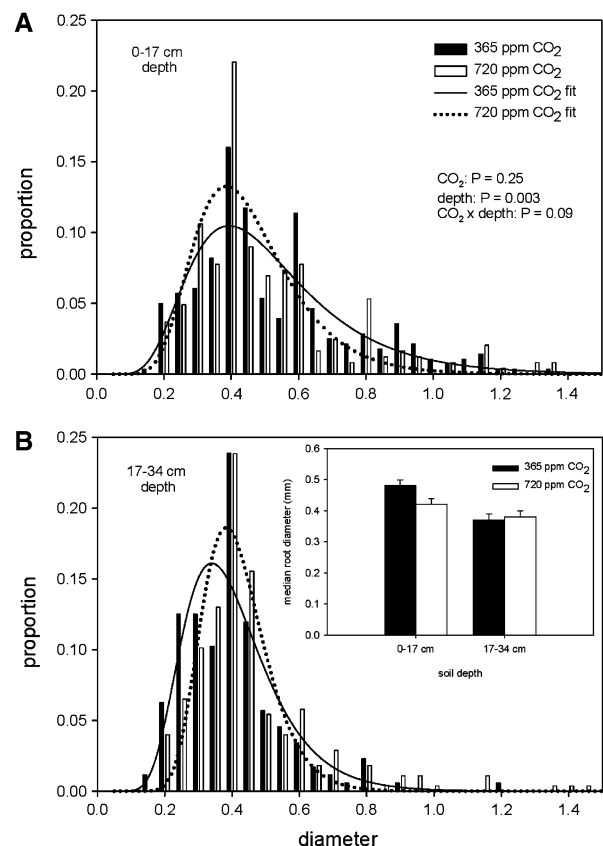

Figure 4. Proportion of fine roots produced at a given diameter under ambient and elevated CO_2 in shallow soil (**A**) and deep soil (**B**). *Inset* shows median root diameter in shallow and deep soil under ambient and elevated CO_2.

during the experiment (Runion and others 2006). Mortality of wiregrass was 97% higher during the course of the experiment in elevated compared to ambient plots. Furthermore, at the plot level, Runion and others (2006) found a 10% increase in total belowground fine root biomass in CO_2-enriched plots, but no change in the biomass of wiregrass fine roots, suggesting a decrease in the proportion of total fine root mass attributed to wiregrass. Furthermore, considered as a proportion of the total root biomass pool, pine roots accounted for 74 and 85% of total belowground biomass in ambient and elevated CO_2 plots, respectively. Allocation to wiregrass roots, on the other hand, decreased from 15% in ambient plots to 6% in CO_2-enriched plots. West and others (2003) examined fine root longevity in wiregrass and reported a lifespan of 777 median days. More recently, Guo and others (2008) reported a median lifespan for longleaf pine of less than 365 days for first-order roots (our most commonly observed root order) and less than 700 days for second-order
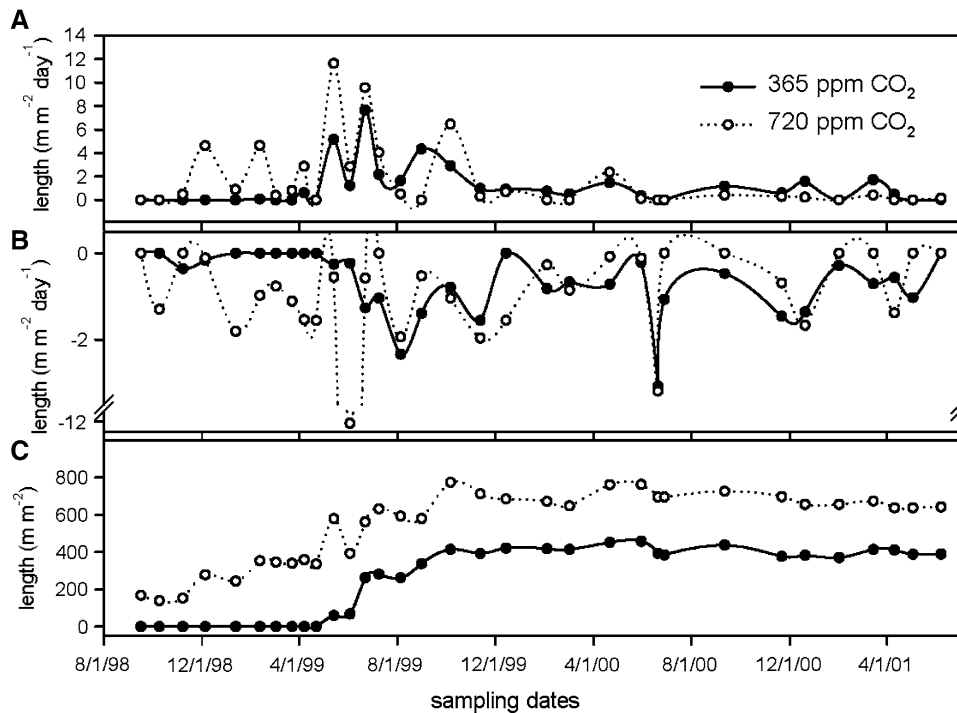
Figure 5. Rhizomorph production (**A**), mortality (**B**), and standing crop (**C**) in the upper soil horizon (0–17 cm) across 33 image collection dates between September 1998 and March 2001.
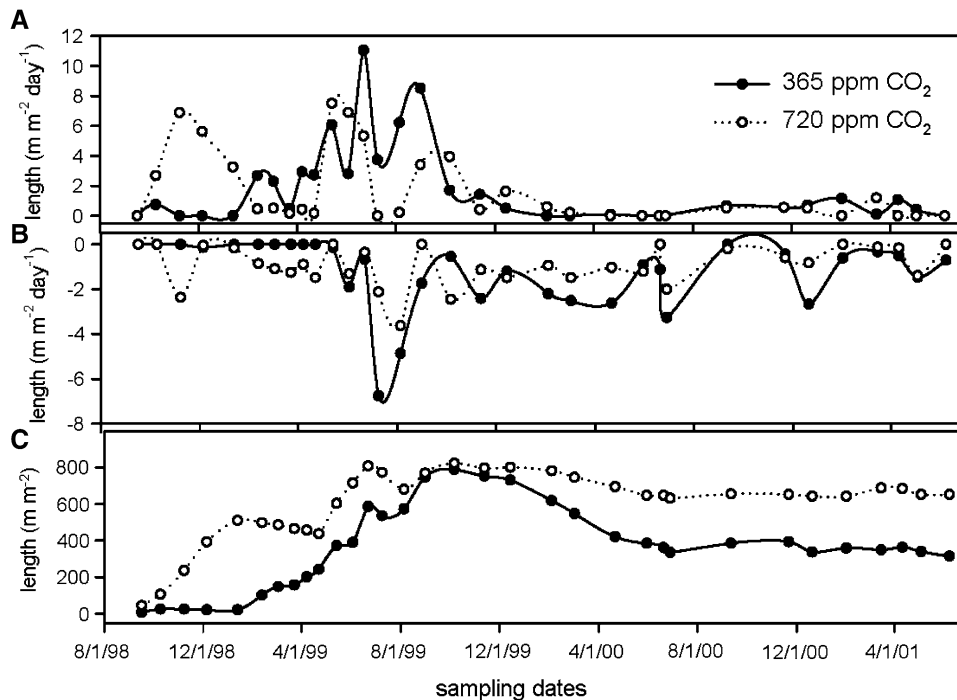

Figure 6. Rhizomorph production (**A**), mortality (**B**), and standing crop (**C**) in the lower soil horizon (17–34 cm) across 33 image collection dates between September 1998 and March 2001.

roots. These results suggest that a lower density and biomass of wiregrass would tend to decrease the average longevity of the fine root pool.

Changes in plant community structure may also have affected the dynamics of soil fungi under elevated CO_2. In particular, the standing crop of rhizomorphs and mycorrhizal tips in the deeper soil horizon increased by 66 and 101%, respectively.

These increases can be tied to the concomitant increase of fine root standing crop in the deeper soil, which likely resulted from increased growth of longleaf pine with elevated CO_2 (Runion and others 2006). The large response by longleaf pine also suggests that increases in rhizomorph and mycorrhizal tip standing crop in deeper soil are attributable to a general increase in mycorrhizal fungi and
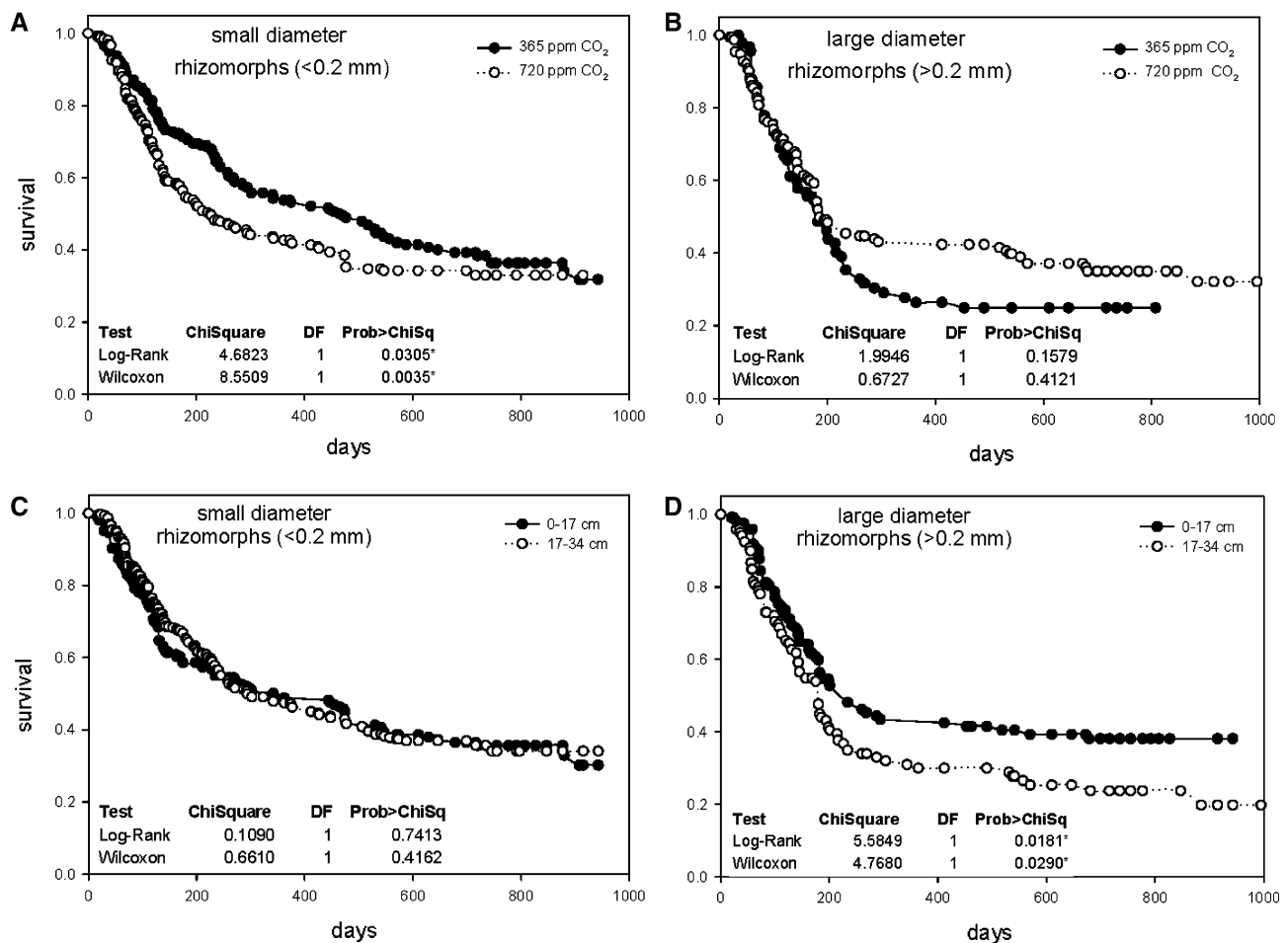
Figure 7. Kaplan Meier survivorship curves displaying interaction of CO_2 on small diameter rhizomorphs (**A**) and large diameter rhizomorphs (**B**) as well as the interaction of depth in soil with small diameter rhizomorphs (**C**) and large diameter rhizomorphs (**D**). Results of Log-Rank and Wilcoxon's tests are shown.

not saprotrophic fungi. Conversely, there was no increase in mycorrhizal tip standing crop or production in the shallow horizon even though rhizomorph standing crop increased significantly. This suggests an increase in the activity of saprotrophic fungi rather than mycorrhizal fungi, perhaps in response to an increase in available litter resulting from the dying leaves and roots of wiregrass, which experienced higher mortality under elevated CO_2. Alternatively, such a shift could also be linked to altered litter quality and/or rhizosphere chemistry in CO_2-enriched plots. An increase in saprotrophic fungi reported here may also partly explain the higher N mineralization rates in elevated CO_2 plots found previously at this site by Torbert and others (2004).

Few studies have addressed rhizomorph survivorship under field conditions (Pritchard and others 2008b; Treseder and others 2005; Vargas and Allen 2008). Overall, results presented here are

similar to those reported by others. Separate from previous studies, we also observed an interesting relationship where rhizomorph survivorship was negatively correlated to diameter (Figure 8). This is contrary to patterns typically observed in fine roots (Wells and Eissenstat 2001). Although it is not surprising that rhizomorphs would exhibit different life history characteristics from fine roots, increasing longevity with decreasing diameter seems counterintuitive from a cost:benefit perspective. As larger diameter rhizomorphs require more C to construct, we expect them to survive longer in order to maximize function while minimizing construction costs. On the other hand, larger structures may cost more to maintain during periods of inactivity or stress. Soil fauna may feed preferentially on rhizomorphs of larger diameter leading to their apparent faster turnover. It may also be that larger diameter rhizomorphs are more common in different species or functional groups of
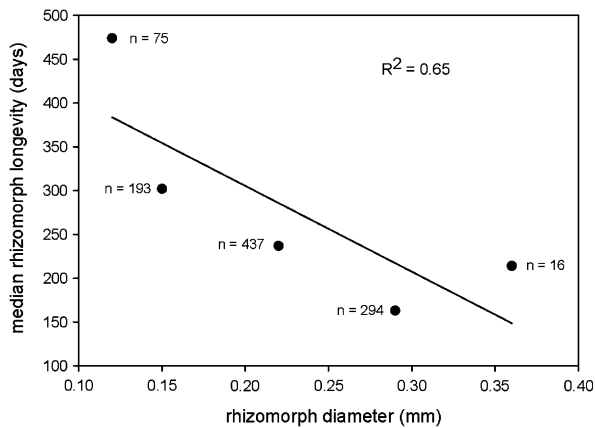
Figure 8. Correlation between rhizomorph diameter and median rhizomorph longevity. Best linear fit ($R^2 = 0.65$).

belowground fungi (for example, mycorrhizal vs. saprotrophic) which may then exhibit different patterns of rhizomorph survivorship, regardless of diameter.

Similar to rhizomorphs, few studies have quantified the survivorship of mycorrhizal tips. We observed a mean mycorrhizal tip survivorship of 159 days across all treatments, which is consistent with other reports (Pritchard and others 2008b). This confirms the common conception that mycorrhizal tips are ephemeral and that their dynamics are likely missed with less frequent sampling.

Elevated CO_2 significantly decreased mean mycorrhizal tip survivorship from 185 to 139 days ($P = 0.008$). Mechanisms to explain this effect include greater flow of carbohydrates belowground, accelerated rate of foraging by mycorrhizal hyphae in nutrient-rich microsites, or possibly increasing production of proteolytic enzymes by the mycorrhizal fungi. Each of these pathways could increase the metabolic activity of the mycorrhizae resulting in faster aging, or increase depletion of nutrient patches. Following patch depletion existing mycorrhizal tips may be abandoned in favor of producing new fungal structures in less depleted patches. Regardless of the driving factors the observed decrease in longevity with elevated CO_2 is consistent with observations from a loblolly pine forest (Pritchard and others 2008b).

Beyond changing uptake and turnover patterns for particular species of soil fungi, increasing the flow of C belowground will also change the function and community composition of fungi. Several studies have demonstrated shifts in fungal communities following tree and whole forest exposure to elevated CO_2 (Godbold and others 1997; Fransson and others 2001; Parrent and Vilgalys 2007).

Furthermore, shifts in species composition will impact decomposition rates of fungal tissues, respiratory response to increasing temperature, as well as the ability of soil fungi to utilize C substrates (Fransson and others 2007; Malcolm and others 2008; Koide and Malcolm 2009). However, each of these studies was limited to species that could be readily cultured in the lab under asymbiotic conditions which only represents a small portion of soil fungi. Clearly, the potential for increased quantity and quality of carbon inputs under CO_2-enriched conditions to significantly alter fungal community composition deserves further study, especially considering the importance of soil fungi for biogeochemical cycling and forest function.

Belowground Productivity and Implications for Nutrient Uptake and Carbon Storage

Elevated CO_2 has been shown to increase standing crop, production, and mortality of fine roots in most tree species and forested ecosystems (Kimball and others 2007; Kubiske and others 1998; Norby and others 2004; Pritchard and others 2008a; Thomas and others 1999; Wan and others 2004) as well as increase mycorrhizal colonization and rhizomorph production (Pritchard and others 2008b; Runion and others 1997; Treseder 2004). Effects of elevated CO_2 may, however, vary with species, study duration, and background soil fertility (Kasurinene and others 1999; Lukac and others 2003; Oren and others 2001). In this study, we observed moderate overall increases in fine roots and larger increases in mycorrhizal tip production with greater increases occurring in deeper soil. Rhizomorph production was increased in both soil horizons.

Plants grown under elevated CO_2 fix more C and can allocate a larger proportion of their carbohydrate budget belowground which facilitates additional soil nutrient uptake. Whether increases in belowground production observed with elevated CO_2 in the current study were proportional to aboveground increases in the current study is equivocal. For example, Runion and others (2006) conducted a destructive harvest at the end of this experiment and found that increases in fine root biomass roughly mirrored increases in aboveground biomass. Similarly, our minirhizotron observations suggested increased allocation to roots early in the study but a dampening of this effect by the end of 2.5 years. On the other hand, significant increases in mycorrhizal tips and rhizomorphs suggest that proportional C allocation belowground exceeded
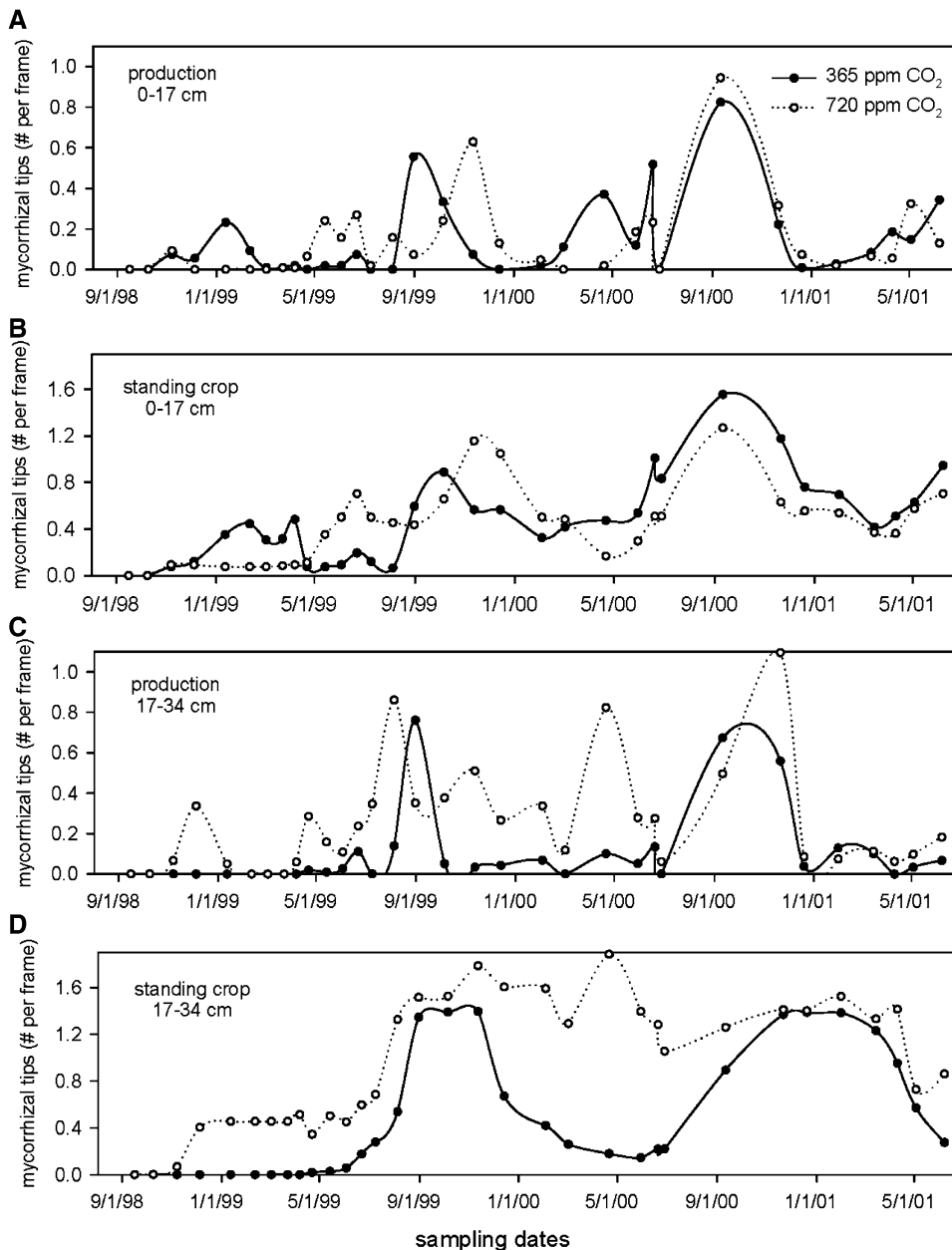
Figure 9. Mycorrhizal tip production and standing crop in shallow soil (0–17 cm) and deep soil (17–34 cm) across 33 image collection dates between September 1998 and March 2001.

that to aboveground biomass during much of the experiment. Our inability to accurately quantify C allocated to mycorrhizal tips, rhizomorphs, and particularly hyphae represents a significant gap in our knowledge of nutrient cycling, C allocation, and ecosystem functioning that must be addressed in future studies.

Fine root, rhizomorph, and mycorrhizal tip production represents a major pathway for C input to soils and has implications for both short- and long-term C storage in forest soils (Harley and Smith 1983; Hobbie and Hobbie 2006; Jackson and others 1997; Marschner and Dell 1994). In this study, we observed significant increases in fine root and

mycorrhizal production with an apparent shift into deeper soils with elevated CO_2. This result is consistent with a number of other forest studies that have also reported higher root density in deep soil with elevated CO_2 (Iversen and others 2008; Norby and others 2004; Pritchard and others 2008a, b; Thomas and others 1999). Shifting allocation to deeper in the soil profile could impact long-term C storage as C in deeper soil turns over (decomposes) more slowly than litter nearer the soil surface (Schöning and Kögel-Knabner 2006; Trumbore 2000). However, more work is needed to determine what proportion of that C simply remains as labile C, with the potential for swift decomposition, and
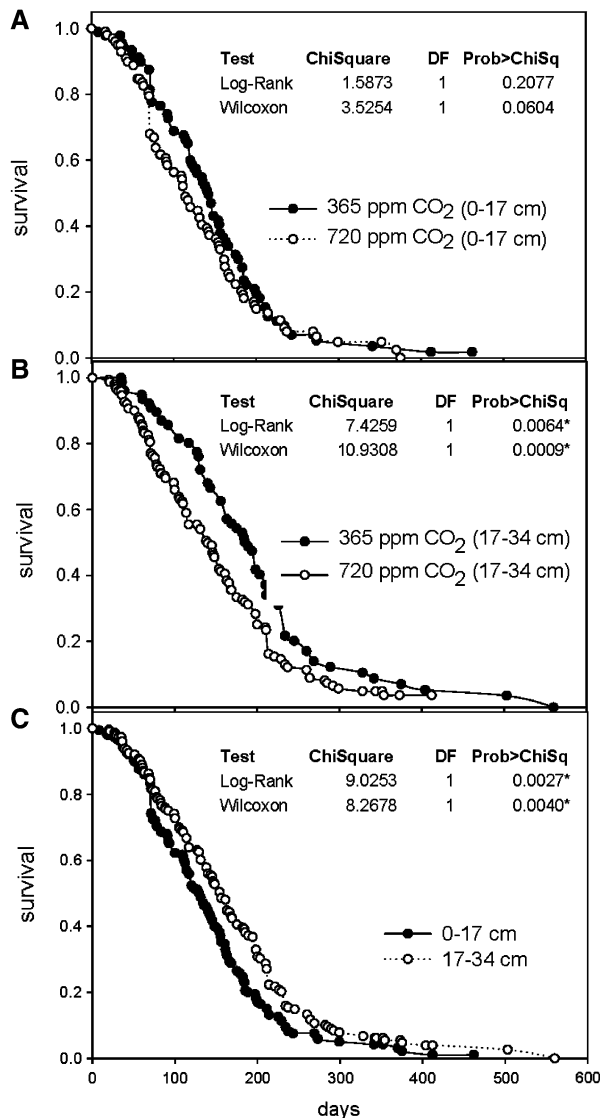
Figure 10. Kaplan–Meier survivorship curves for mycorrhizal root tips in elevated and ambient plots in shallow (**A**) and deep (**B**) soil. Also shown is the main effect of depth on survivorship (**C**). Results of Log-Rank and Wilcoxon's tests are shown.

how much C will enter a more recalcitrant soil C pool. Regardless, if such a response proves to be consistent across forest types and ages, then greater biomass production in deeper soils in the coming decades has the potential to contribute to greater C storage in forest soils and has similarly important implications for nutrient cycling (Iversen and others 2008). Furthermore, fungal tissues consist largely of chitin, a potentially recalcitrant compound that may build up SOM and persist for long periods of time relative to more labile C containing compounds such as root exudates and fine roots themselves (Langley and Hungate 2003). Colonization by

ectomycorrhizal fungi may also retard decomposition of fine roots as they are somewhat shielded from outside decomposers by the dense fungal mantle and the hartig net. Finally, ectomycorrhizas may also reduce the ability of saprotrophic fungi to decompose SOM via the Gadgil effect whereby ectomycorrhizal fungi compete with saprotrophic fungi for soil resources (Gadgil and Gadgil 1975; Koide and Wu 2003; Langley and others 2006).

Alternatively, increased production of roots and fungi may accelerate the breakdown of recalcitrant soil organic matter (SOM) leading to greater nitrogen uptake but less soil C retention (Read and Perez-Moreno 2003). Such a "priming" effect is likely responsible for the increase in mineralization rates and lack of C storage noted in other CO_2-enrichment experiments (Kuzyakov and others 2000; Carney and others 2007). Unfortunately, without knowing how local environmental and climatic factors will change over the next decades it is difficult to predict exactly how mycorrhizal fungi will affect long-term soil C storage (Talbot and others 2008).

CONCLUSIONS

Our findings suggest that regenerating longleaf pine-wiregrass systems may act as a C sink as atmospheric CO_2 rises in the coming decades through increased biomass production and potentially through directed allocation of C to deeper soils. This will be of particular importance as ecological restoration efforts return more land to longleaf habitat and these systems play a larger role in the overall C budget of the Southeastern United States (Van Lear and others 2005). The results presented here are also consistent with the recent assertion that greater allocation of forest C to deeper soil is a general response to atmospheric CO_2-enrichment (Iversen 2010). The stimulation in production and shift of fine roots and mycorrhizae into deeper soil will increase the total input of C to the deeper soil horizons, although the fate of that C is still largely unclear and will depend in part on whether mycorrhizal fungi and other soil microbes significantly increase the breakdown of SOM throughout the soil profile.

Our results emphasize the importance of considering the role of both fine roots and associated fungi in mediating tree and forest response to atmospheric CO_2-enrichment. Results of this experiment indicated that soil fungi were influenced more by elevated atmospheric CO_2 than were fine roots. It is not known if this represents a general ecosystem response to elevated CO_2 because fine roots and associated fungal structures

have rarely been considered within the same experiment. The extent to which roots and different functional groups of soil fungi respond to global changes are likely to influence the capacity of ecosystems to exploit additional atmospheric CO_2 in both the short- and long-term. For instance, significant increases in mycorrhizae and rhizomorphs as reported here may explain why the magnitude of the increase in forest NPP caused by elevated CO_2, in several long-term demonstrably N-limited FACE (Free Air CO_2-Enrichment) experiments, has not decreased after nearly a decade (Finzi and others 2007).

We caution, however, that results from studies on simplified ecosystems, particularly those in the early stages of development, may not apply to natural, more mature forest stands. Projections of the fate of ecosystems dominated by longleaf pine and wiregrass are further complicated by the important role played by fire in influencing biogeochemistry and shaping plant species composition. Finally, it is also important to recognize that the relative importance of roots and soil fungi in mediating ecosystem response to rising atmospheric CO_2 will likely depend on other global change factors such as increasing temperature and changing precipitation regimes. As such more long-term studies investigating interactions between different global change factors with particular emphasis on belowground dynamics in different ecosystems are needed.

ACKNOWLEDGMENTS

REFERENCES

Carney KM, Hungate BA, Drake BG, Megonigal JP. 2007. Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. PNAS 104:4990–5.

Davis MA, Pritchard SG, Mitchell RJ, Prior SA, Rogers HH, Runion GB. 2002. Elevated atmospheric CO_2 affects structure of a model regenerating longleaf pine community. J Ecol 90:130–40.

Dixon RK, Solomon AM, Brown S, Houghton RA, Trexier MC, Wisniewski J. 1994. Carbon pools and flux of global forest ecosystems. Science 263:185–90.

Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL. 2000. Building roots in a changing environment: implications for root longevity. New Phytol 147:33–42.

Finzi AC, Norby RJ, Calfapietra C, Gallet-Budynek A, Gielen B, Holmes WE, Hoosbeek MR, Iversen CM, Jackson RB, Kubiske ME, Ledford J, Liberloo M, Oren R, Polle A, Pritchard S, Zak DR, Schlesinger WH, Ceulemans R. 2007. Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO_2. PNAS 104:14014–19.

Fransson PM, Taylor AFS, Finlay RD. 2001. Elevated atmospheric CO_2 alter root symbiont community structure in forest trees. New Phytol 152:431–42.

Fransson PMA, Anderson IC, Alexander IJ. 2007. Ectomycorrhizal fungi in culture respond differently to increased carbon availability. FEMS Microbiol Ecol 61:246–57.

Friedlingstein P, Cox P, Betts R, Bopp L, Von Bloh W, Brovkin V, Cadule P, Doney S, Eby M, Fung I, Bala G, John J, Jones C, Joos F, Kato T, Kawamiya M, Knorr W, Lindsay K, Matthews HD, Raddatz T, Rayner P, Reick C, Roeckner E, Schnitzler KG, Schnur R, Strassmann K, Weaver AJ, Yoshikawa C, Zeng N. 2006. Climate carbon cycle feedback analysis: results from the C^4MIP model intercomparison. J Clim 19:3337–53.

Gadgil RL, Gadgil PD. 1975. Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. New Zealand J For Sci 5:33–41.

Godbold DL, Berntson GM, Bazzaz FA. 1997. Growth and mycorrhizal colonization of three North American tree species under elevated atmospheric CO_2. New Phytol 137:433–40.

Goodale CL, Apps MJ, Birdsey RA. 2002. Forest carbon sinks in the northern hemisphere. Ecol Appl 12:891–9.

Guo D, Mitchell RH, Withington JM, Fan P-P, Hendricks JJ. 2008. Endogenous and exogenous controls of root life span, mortality and nitrogen flux in a longleaf pine forest: root branch order predominates. J Ecol 96:737–45.

Harley JL, Smith SE. 1983. Mycorrhizal symbiosis. London: Academic Press.

Hobbie JE, Hobbie EA. 2006. 15 N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in arctic tundra. J Ecol 87:816–22.

Iversen CM. 2010. Digging deeper: fine-root responses to rising atmospheric CO_2 concentration in forested ecosystems. New Phytol 186:346–57.

Iversen CM, Ledford J, Norby RJ. 2008. CO_2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. New Phytol 179:837–47.

Jackson RB, Mooney HA, Schulze E-D. 1997. A global budget for fine root biomass, surface area, and nutrient contents. PNAS 94:7362–6.

Johnson MG, Meyer PF. 1998. Mechanical advancing handle that simplifies minirhizotron camera registration and image collection. J Environ Qual 27:710–14.

Kaplan EL, Meier P. 1958. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–81.

Kasurinene AE, Helmisaari H-S, Holopainen T. 1999. The influence of elevated CO_2 and O_3 on fine roots and mycorrhizas of naturally growing young Scots pine trees during three exposure years. Glob Change Biol 5:771–80.

Kimball BA, Idso SB, Johnson S, Rillig MC. 2007. Seventeen years of carbon dioxide enrichment of sour orange trees: final results. Glob Change Biol 13:1–13.

Koide RT, Malcolm GM. 2009. N concentration controls decomposition rates of different strains of ectomycorrhizal fungi. Fungal Ecol 2:197–202.

Koide RT, Wu T. 2003. Ectomycorrhizas and retarded decomposition in a *Pinus resinosa* plantation. New Phytol 158:401–7.

Kubiske M, Pregitzer KS, Zak DR, Mikan CJ. 1998. Growth and C allocation of *Populus tremuloides* genotypes in response to atmospheric CO$_2$ and soil N availability. New Phytol 140:250–1.

Kuzyakov Y, Friedel JK, Stahr K. 2000. Review of mechanisms and quantification of priming effects. Soil Biol Biogeochem 132:1485–98.

Lal R. 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123:1–22.

Lal R. 2005. Forest soils and carbon sequestration. For Ecol Manag 220:242–58.

Langley JA, Hungate BA. 2003. Mycorrhizal controls on belowground litter quality. J Ecol 84:2302–12.

Langley JA, Chapman SK, Hungate BA. 2006. Ecotomycorrhizal colonization slows root decomposition: the *post-mortem* fungal legacy. Ecol Lett 9:955–9.

Lukac M, Calfapietra C, Godbold DL. 2003. Production turnover and mycorrhizal colonization of root systems of three *Populus* species grown under elevated CO$_2$ (POPFACE). Glob Change Biol 9:838–48.

Malcolm GM, Lopez-Gutierrez JC, Koide RT, Eissenstat DM. 2008. Acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi. Glob Change Biol 14:1169–80.

Marschner H, Dell B. 1994. Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102.

Meehl GA, Stocker TF, Collins WD and others. 2007. Global climate projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, Eds. Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge, UK and New York (NY), USA: Cambridge University Press.

Milchunas DG, Morgan JA, Mosier AR, Lecain DR. 2005. Root dynamics and demography in shortgrass steppe under elevated CO$_2$, and comments on minirhizotron methodology. Glob Change Biol 11:1837–55.

Mitchell RJ, Runion GB, Prior SA, Rogers HH, Amthor JS, Henning FP. 1995. Effects of nitrogen on *Pinus palustris* foliar respiratory responses to elevated atmospheric CO$_2$ concentration. J Exp Bot 46:1561–7.

Norby RJ, Jackson RB. 2000. Root dynamics and global change: seeking an ecosystem perspective. New Phytol 147:3–12.

Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EF. 2004. Fine-root production dominates response of a deciduous forest to atmospheric CO$_2$ enrichment. PNAS 101:9689–93.

Oren R, Ellsworth DS, Johnsen KH. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO$_2$-enriched atmosphere. Nature 411:469–72.

Parrent JL, Vilgalys R. 2007. Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO$_2$ and nitrogen fertilization. New Phytol 176:164–74.

Pritchard SG, Davis MA, Mitchell RJ, Prior SA, Boykin DL, Rogers HH, Runion GB. 2001. Root dynamics in an artificially constructed regenerating longleaf pine ecosystem are affected by atmospheric CO$_2$ enrichment. Environ Exp Bot 46:55–69.

Pritchard SG, Strand AE, McCormack ML et al. 2008a. Fine root dynamics in a loblolly pine forest are influenced by free-air-CO$_2$-enrichment: a six-year-minirhizotron study. Glob Change Biol 14:588–602.

Pritchard SG, Strand AE, McCormack ML, Davis MA, Oren R. 2008b. Mycorrhizal and rhizomorph dynamics in a loblolly pine forest during five years of Free-Air CO$_2$-enrichment (FACE). Glob Change Biol 14:1252–64.

Read DJ, Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems-a journey towards relevance? New Phytol 157:475–92.

Richter DD, Markewitz D, Trumbore SE, Wells CG. 1999. Rapid accumulation and turnover of soil carbon in a re-establishing forest. Nature 400:56–8.

Rillig MC, Field CB, Allen MF. 1999. Soil biota responses to long-term atmospheric CO$_2$ enrichment in two California annual grasslands. Oecologia 119:572–7.

Runion GB, Mitchell RJ, Rogers HH, Prior SA, Counts TK. 1997. Effects of nitrogen and water limitation and elevated CO$_2$ on ectomycorrhizal of longleaf pine. New Phytol 137:681–9.

Runion GB, Davis MA, Pritchard SG, Prior SA, Mitchell RJ, Torbert HA, Rogers HH, Dute RR. 2006. Effects of elevated atmospheric carbon dioxide on biomass and carbon accumulation in a model regenerating longleaf pine community. J Environ Qual 35:1478–86.

Schimel DS, House JI, Hibbard KA, Bousquet P, Ciais P, Peylin P, Braswell BH, Apps MJ, Baker D, Bondeau A, Canadell J, Churkina G, Cramer W, Denning AS, Field CB, Friedlingstein P, Goodale C, Heimann M, Houghton RA, Melillo JM, Moore B, Murdiyarso D, Noble I, Pacala SW, Prentice IC, Raupach MR, Rayner PJ, Scholes RJ, Steffen WL, Wirth C. 2001. Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. Nature 414:169–72.

Schöning I, Kögel-Knabner I. 2006. Chemical composition of young and old carbon pools throughout Camisol and Luvisol profiles under forests. Soil Biol Biochem 38:2411–24.

Steel RGD, Torrie JH, Dickey DA. 1997. Principles and procedures of statistics: a biometrical approach. 3rd ed. New York: McGraw-Hill.

Talbot JM, Allison SD, Treseder KK. 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Funct Ecol 26:955–63.

Thomas SM, Whitehead D, Reid JB, Cook FJ, Adams JA, Leckie AC. 1999. Growth, loss, and vertical distribution of *Pinus radiata* fine roots growing at ambient and elevated CO$_2$ concentration. Glob Change Biol 5:107–21.

Torbert HA, Prior SA, Runion GB, Davis MA, Pritchard SG, Rogers HH. 2004. Nitrogen and carbon cycling in a model longleaf pine community as affected by elevated atmospheric CO$_2$. J Environ Manag 33:S132–8.

Treseder KK. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus and atmospheric CO$_2$ in field studies. New Phytol 164:347–55.

Treseder KK, Allen MF, Ruess RW, Pregitzer KS, Hendrick RL. 2005. Lifespans of fungal rhizomorphs under nitrogen fertilization in a pinyon-juniper woodland. Plant Soil 270:249–55.

Trumbore S. 2000. Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecol Appl 10:399–411.

Van Lear DH, Carroll WD, Kapeluck PR, Johnson R. 2005. History and restoration of the longleaf pine-grassland ecosystem: implication for a species at risk. For Ecol Manag 211:150–75.

Vargas R, Allen MF. 2008. Dynamics of fine root, fungal rhizo-morphs, and soil respiration in a mixed temperate forest: Integrating sensors and observations. Vadose Zone J 7:1055–64.

Vogt KA, Vogt DJ, Gower ST, Grier CC. 1990. Carbon and nitrogen interactions for forests ecosystems. In: P. H. Commission of the European Communities, Ed. Air pollution report 32. Environment Research Programme. Above- and below-ground nitrogen interactions in forest trees in acidified soils. Brussels: Directorate-General for Science, Research and Development. pp 203–35.

Wan S, Norby RJ, Pregitzer KS, Ledford J, O'Neill EG. 2004. CO_2 enrichment and warming of the atmosphere enhance both productivity and mortality of maple tree fine roots. New Phytol 162:437–46.

Wells CE, Eissenstat DM. 2001. Marked differences in survivorship among apple roots of different diameters. J Ecol 82:882–92.

West JB, Espeleta JF, Donovan LA. 2003. Root longevity and phenology differences between two co-occurring savanna bunchgrasses with different leaf habits. Funct Ecol 17:20–8.